SEPARATUM • S. KARGER • BASEL–NEW YORK • Printed in Switzerland

Int. Arch. Allergy 27: 35–45 (1965).

Role of Hyperreactivity of Bronchial and Pulmonary Vascular Muscle to Acetylcholine and Histamine in Anaphylactic Shock in Rabbits and Guinea-Pigs*

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Liberation of excessive amounts of acetylcholine (ACh) has been considered by Daniélopolu (1 to 3) as the dominant factor in triggering pathogenic mechanisms in anaphylactic shock (AS), serum sickness, asthma and other pathological conditions associated with antigen-antibody reactions. In agreement with Daniélopolu's hypothesis, Wenner and Buhrmester (4) reported an increase of ACh in the blood of sensitized and shocked rabbits. However, Kourilsky, Guillot and Gwan (5) and Ratnoff (6) questioned the importance of ACh in AS because they failed to detect increased amounts of ACh in shocked tissues or in the body fluids of various shocked animals.

Failure to detect excessive amounts of ACh would not preclude the hypothesis that ACh plays a dominant role in the pathogenesis of AS if we assume that the pathogenic factor is hyperreactivity to amounts of ACh normally present in tissues, rather than liberation of an excess of ACh. Hyperreactivity of pulmonary vascular muscle to small doses of ACh can be induced by an extrinsic agent such as endotoxin (7), and its role in the pathogenesis of endotoxin shock has been investigated (8). Hyperreactivity of bronchial smooth muscle to ACh and, to a lesser degree, to histamine, has been reported in asthmatic patients (9 to 10).

* This work was sponsored by the Office of Naval Research and the Bureau of Medicine and Surgery, United States Navy, under a contract between the Office of Naval Research and the Regents of the University of California. Reproduction in whole or in part is permitted for any purpose of the United States Government.

In the present work we investigated whether hyperreactivity of pulmonary vascular and bronchial muscle to circulating ACh could be demonstrated in AS of rabbits and guinea-pigs. Responsiveness to histamine during AS was also investigated, since a major role in the pathogenesis of AS is currently ascribed to this substance.

Materials and Methods

Experimental animals. Female New Zealand white rabbits, weighing 2.1 to 2.9 kg, and male and female guinea-pigs, weighing from 490 to 650 g, were used.

Anesthetics and anticoagulant. Sodium phenobarbital (80 mg/kg) and sodium pentobarbital (16 mg/kg) were injected intravenously (i.v.) into rabbits. The guinea-pigs were anesthetized with intraperitoneal (i. p.) injections of 39 to 48 mg sodium pentobarbital/kg. Heparin, in doses of 1500 units/kg, was injected i. v. into rabbits and guinea-pigs before challenge with antigen.

Sensitization. Fresh albumen diluted 1 : 2 with physiological saline was used as sensitizing antigen. Fourteen rabbits and seven guinea-pigs were sensitized on 3 consecutive days with i. p. injections of 2 ml and 0.2 ml/animal respectively.

Challenge. The animals were challenged 21 to 31 days after the first sensitizing injection with 1 i. v. injection of fresh albumen. The injection was administered in less than 2 min. We attempted to induce only a moderate shock by varying the challenge dose from 1 ml of a 1 : 2 solution to 0.03 ml of a 1 : 20 solution/kg for the rabbits, and from 0.7 ml of a 1 : 2 solution to 0.1 ml of a 1 : 15 solution/kg for the guinea-pigs. The animals that survived the anaphylactic reaction were challenged again with the same, or a larger, dose of albumen.

Parameters. Mean systemic arterial pressure (Ap) was recorded from the femoral artery in rabbits and from the left carotid artery in guinea-pigs. Right ventricular pressure (RVp) was recorded in both species from the right ventricle cannulated from the right jugular vein. In some animals central venous pressure was recorded from the superior vena cava. Intratracheal pressure (ITp) was determined in those animals in which artificial positive-pressure respiration was used for ventilation of the lungs. The thorax was kept wide open and the respiratory rate and volume were maintain-

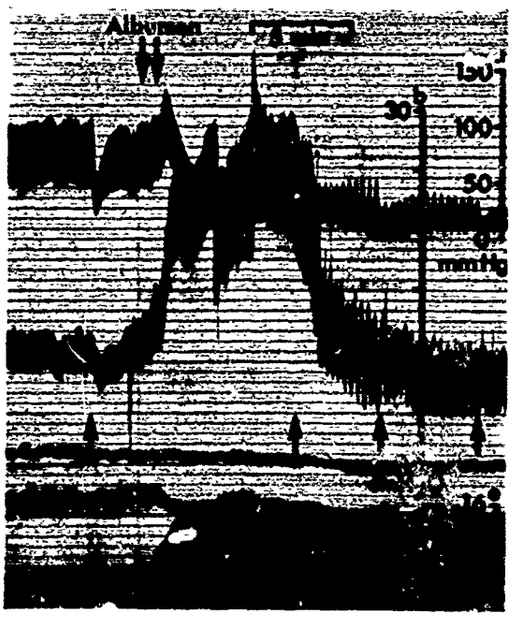

Fig. 1. Response to intravenous injection of acetylcholine in systemic arterial pressure (a), right ventricular pressure (b), central venous pressure (c), left atrial pressure (d), and intratracheal pressure (e) during anaphylactic shock in rabbit No. 8 (table 1), sensitized 21 days prior to challenge. The animal expired 47 min after challenge.

Two arrows pointing downward indicate beginning and end of injection of 0.1 ml of a 1 : 4 solution of albumen/kg. Arrow pointing upward indicates injection of 0.3 μg acetylcholine/kg.

ed constant throughout the experiment. In some animals, the left atrium was cannulated. All pressures were transmitted to Statham pressure transducers and recorded with a Honeywell Visicorder.

Drugs. ACh and histamine, in constant concentration (10μg/ml), were infused into the left jugular vein at constant rates. Duration of infusion varied according to desired dose. Injections were given at intervals of approximately 2 to 10 min before and during AS, and in animals that survived AS, for a minimum period of 40 min after shock.

Control injections of endotoxin. Injections of endotoxin (25 to 50 μg/kg) were made in the jugular vein of 5 rabbits that had recovered from anaphylactic reaction to test their capacity to develop hyperreactivity to ACh in the pulmonary circulation.

Results

Among the 14 rabbits and 7 guinea-pigs which received challenge injections of albumen, 7 of the rabbits and all guinea-pigs died within 7 to 18 min. These results, along with the magnitude of changes in the various parameters which were studied, are shown in tables I and II and exemplified in fig. 1 and 2.

These data show that AS was characterized in all rabbits by a severe fall in Ap (table I, fig. 1 and 2). In 10 of the 14 rabbits, a short rise in Ap (6 to 35% of control values) developed within 0.5 to 4 minutes before the fall in Ap. The minimum in Ap was attained 5 to 18 minutes after challenge. In the rabbits that survived, Ap

Table I

Magnitude of changes in systemic mean arterial pressure (Ap), right ventricular pressure (RVp) and intratracheal pressure (ITp) during anaphylactic shock (AS) in rabbits and guinea-pigs.

Animal No.[1]	Challenge dose of albumen ml/kg (undiluted)	Control values			Maximum changes during AS[2]		
		Ap mm Hg	RVp mm H_2O	ITp cm H_2O	Ap % of control	RVp % of control	ITp % of control
1[3]	0.42	87	124		—100	+143	
2	0.4	100	125		— 55	+152	
3[3]	0.22	43	85	15.2	—100	+100	+21
4	0.14	82	158	15.2	— 64	+131	+15
5[3]	0.1	87	128		—100	+112	
6	0.09	97	120		— 58	+ 69	
7	0.09	100	120		— 60	+175	
8[3]	0.02	78	120	14.0	—100	+191	+12
9[3]	0.01	70	160	14.0	—100	+112	+35
10[3]	0.006	40	120	14.8	—100	+ 3	+37
11[3]	0.004	37	120	14.4	—100	+ 31	+26
12	0.003	55	160	14.0	— 41	— 25	nc
13	0.001	75	190	14.8	— 50	+ 5	+21
14	0.001	85	128	14.8	— 40	+ 15	+ 2
15[3]	0.31	60	158	15.8	+ 73	+ 41	+10
16[3]	0.27	43	80	13.8	+151	+125	+30
17[3]	0.21	27	75	15.7	+100	+ 36	+ 3
18[3]	0.15	25	140	9.0	+336	+157	+55
19[3]	0.07	20	120		+175	+ 16	
20[3]	0.02	35	93	14.0	+228	+109	+37
21[3]	0.004	28	92	18.9	+267	+106	+17

[1] Animals No. 1 to 14: rabbits; No. 15 to 21: guinea-pigs.

[2] Maximum changes recorded after injection of challenge dose of albumen; +: increase in pressure; —: fall in pressure; nc: no change.

[3] Shock was followed by death.

Table II

Response in systemic mean arterial pressure(Ap), systolic right ventricular pressure (RVp), and intratracheal pressure (ITp) to i. v. injection of acetylcholine (ACh) and histamine during anaphylactic shock in rabbits and guinea-pigs

Animal No.[1]	ACh µg/kg	Response to acetylcholine[2]						Response to histamine[2]					
		Before shock			During shock			Histamine, µg/kg	Before shock			During shock	
		Ap mm Hg	RVp mm H_2O	ITp mm H_2O	Ap mm H_2O	RVp mm H_2O	ITp mm H_2O		Ap mm Hg	RVp mm H_2O	ITp mm H_2O	Ap mm Hg	RVp mm H_2O	ITp mm H_2O
1	0.8	—42	—16		nc	nc		8.3	— 9	+21		nc	+ 5	
2	0.8	—33	— 5		—15	nc		4.0	—17	+ 2		nc	nc	
4	1.0	—40	—25	+ 8	—37	—55	nc	4.7	—15	+14	nc	nc	+ 5	nc
								4.7				nc	+12	nc
5	3.2	—59	—10		—30	—80		0						
6	0.8	—24	— 8		—26	—36		3.6	— 9	+ 5		nc	nc	
	1.7	—24	—10		—27	—44		3.6				nc	nc	
7	0.1	—43	—16		—18	—44		4.5	— 6	— 7		nc	nc	
								9.0	—15	+12		— 5	+14	
8	0.3	—43	—24	nc	— 7	nc	nc	0						
	0.3				— 9	nc	nc							
	0.3				—12	nc	nc							
11	0.3	—25	—10	nc	—25	—10	nc	0						
	0.3				—15	nc	nc							
12	0.2	—35	—15	nc	—15	—20	nc	0						
14	0.4	—57	—10	nc	—30	—15	nc	0						
					—40	— 5	nc							
15	3.0	—19	—47	nc	nc	nc	nc	3.0	— 6	—10	nc	nc	nc	nc
								6.0	—13	—15	+ 8	nc	nc	nc
16	3.5	—10	— 5	nc	nc	nc	nc	3.5	— 9	+ 2	nc	nc	nc	nc
	3.5				nc	nc	nc							
17	3.3	— 8	—13	nc	nc	nc	nc	0						
18	3.3	—11	—15	nc	nc	nc	nc	6.7	—21	+ 5	+18	nc	nc	nc
19	3.5	—11	—20	+ 20	— 6	— 3	nc	7.0	nc	—11	+20	—15	— 7	nc
	3.5				— 3	nc	nc	7.0			nc	nc	nc	nc
20	3.7	—20	—12	nc	— 8	—25	nc	7.4	— 9	—10		nc	nc	nc
								7.4				nc	nc	nc
21	4.9	—13	—10	nc	—48	—25	nc	9.2	— 9	+ 20	+10	—15	+22	nc
	4.0				—19	—20	nc	9.2				—11	nc	nc
								9.2				nc	nc	nc

[1] No. 1 to 14: rabbits; No. 15 to 21: guinea-pigs; animal numbers correspond to numbers in table I.

[2] + : rise in pressure; — : fall in pressure; nc: no change.

recovered 53 to 100% of control values within 14 to 29 min after challenge(fig. 2). In all guinca-pigs, a pronounced rise in Ap (73 to 336% of control values) (table I) developed immediately after challenge and was followed 3 to 8 min later by an abrupt fall to 0 mm Hg and death.

Approximately the same proportion of animals in both species reacted to challenge with a marked rise in RVp that preceded the changes in Ap. Five rabbits that had received challenge doses of albumen smaller than 0.01 ml/kg reacted with only a minor rise or even a fall in RVp (tableI). Four of the nine rabbits that reacted with a marked rise in RVp survived, while two of the five rabbits that did not respond with a significant rise in RVp expired (table I).

Rise in ITp (table I) developed immediately after challenge in the guinea pigs and reached maximum values abruptly without any further significant change until death. In the rabbits that died, rise in ITp also began immediately after injection of albumen and reached its maximum 2 to 6 min later without any significant decrease until death (fig. 1). Among the 4 survivors in which ITp was recorded, 2 animals responded with an increase in ITp of 15 and 21% of the control values that persisted while we observed a recovery in Ap. In the other 2 surviving rabbits there was either no change in ITp or a small transient increase. The magnitude of changes in RVp and ITp was comparable in both species. A correlation between the magnitude of changes in Ap, RVp and ITp or between the magnitude of changes in these parameters and the incidence of death could not be established in either rabbits or guinea-pigs.

Responsiveness to ACh and to histamine during AS in rabbits. Injections of ACh in doses of 0.1 to 3.2 μg/kg before challenge resulted in a fall in Ap of 24 to 59 mm Hg (table II). Injections of the same doses during the acute phase of shock resulted in a fall in Ap of 7 to 40 mm Hg in 9 animals and no change in 1 animal. Control injections of ACh elicited a fall in RVp of 5 to 25 mm H_2O. During AS, ACh elicited no response in RVp in 3 animals and a fall of 5 to 80 mm H_2O in 7 animals. We did not observe a pressor response in any of the animals. The duration of the response in Ap and RVp during AS did not differ from the duration of response recorded before challenge. The response of animals to ACh in Ap and in RVp after their recovery from acute shock was similar to the response observed before challenge.

Five of 14 rabbits received injections of histamine. Doses of 3.6 to 9.0 μg/kg before challenge resulted in a fall in Ap of 6 to 17 mm Hg (table II). Injections of the same doses during the acute phase of shock elicited a fall of 5 mm Hg in 1 animal and no change in 4 animals. The response in RVp to histamine before challenge varied from a fall of 7 to a rise of 21 mm H_2O (table II). During AS there was no response in 2 animals and a rise of 5 to 14 mm H_2O in 3 animals. The duration of response before and during shock was the same. We observed no significant change in response of ITp to ACh or to histamine during AS.

Responsiveness to ACh and histamine during AS in guinea-pigs. Injections of ACh in doses of 3.0 to 4.0 μg/kg before challenge resulted in a fall in Ap of 8 to 20 mm Hg (table II). Injections of the same doses during AS elicited no response in 4 animals and a fall of 3 to 48 mm Hg in 3 animals. Injections of ACh caused a fall in RVp of 5 to 47 mm H_2O before challenge and a fall of 3 to 25 mm H_2O in 3 animals or no change in 4 animals during AS (table II). The duration of response both in Ap and in RVp was similar before and during shock. We observed no change in the response of ITp to ACh during AS.

Injections of histamine in doses of 3.0 to 9.2 μg/kg before challenge resulted in a fall in Ap of 6 to 21 mm Hg in 5 animals and no change in 1 animal (table II). During AS, the injections produced no appreciable effect in 4 animals and a fall of 11 to 15 mm Hg in 2 animals. The response in RVp to histamine varied from a fall of 15 to a rise of 20 mm H_2O before challenge and a fall of 7 to a rise of 22 mm H_2O during AS in 2 animals, and there was no change in the other 4 animals. No appreciable change in duration of response in Ap and in RVp was observed during AS. There was a small response in ITp to injections of histamine before challenge in 4 of 6 animals (table II), but no change was observed during AS.

Responsiveness to ACh following injection of endotoxin in rabbits. Five of the seven rabbits that had recovered from AS, and had become tachyphylactic to further injections of albumen, received endotoxin. Endotoxin was injected 16 to 41 min after the last injection of albumen. Pre-endotoxin injections of ACh elicited a fall in RVp of 5 to 25 mm H_2O. Post-endotoxin injections of ACh produced a pressor response in RVp of 8 to 32 mm H_2O, 20 to 30 min after injection of endotoxin. The response increased also in duration. In one

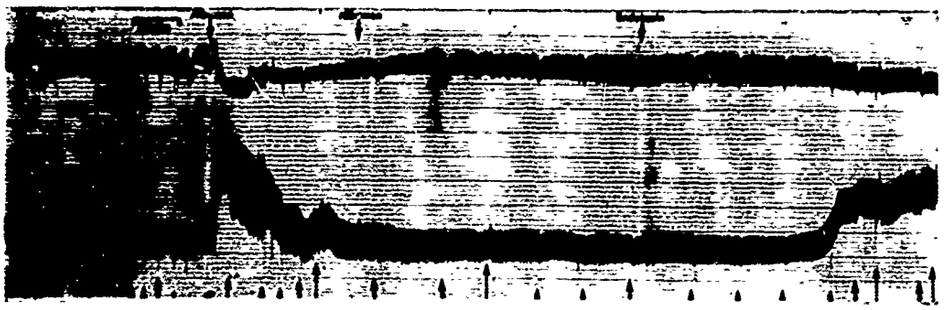

Fig. 2. Response to intravenous injection of acetylcholine and of histamine in systemic arterial pressure (a) and right ventricular pressure (b) during anaphylactic reaction and following injection of *Pseudomonas pseudomallei* endotoxin in rabbit No. 2 (table I), sensitized 21 days prior to challenge.

Arrow pointing downward indicates injection of albumen (0.8 ml of a 1 : 2 solution/kg) or injection of endotoxin (250 μg/kg). Arrows pointing upward and graduated in length indicate injection of 0.8 and 1.6 μg acetylcholine/kg respectively. Dotted arrows pointing upward and graduated in length indicate injection of 4.0 and 8.0 μg histamine/kg respectively.

experiment (fig. 2), injection of ACh 25 min after administration of endotoxin elicited a sustained rise in RVp of 150 mm H_2O.

Discussion

The predominant difference observed in our experiments between AS in rabbits and AS in guinea-pigs was a pronounced fall in Ap in rabbits and a marked rise in Ap in guinea-pigs following challenge with albumen.

The lack of correlation between the magnitude of rise in RVp and the magnitude of fall in Ap or incidence of death in AS in rabbits does not support the current theory (11) that pulmonary vasoconstriction resulting in reduced filling of the left ventricle is the primary cause of death in rabbits. Coronary vasospasm resulting in cardiac failure may have been the cause of the abrupt fall in Ap in AS of rabbits (11).

While injections of doses of antigen smaller than 0.01 ml/kg (table I) in rabbits were followed by a less pronounced change in RVp, the magnitude of fall in Ap was not significantly affected. The number of experiments does not allow us to conclude that rise in RVp during AS in rabbits is observed only with doses of antigen

that exceed a certain threshold dose, while fall in Ap can be elicited even with smaller doses of antigen.

ACh did not elicit a pressor response in RVp during AS in any of the animals. The rabbits that received endotoxin after recovery from AS responded to circulating ACh with a rise in RVp 20 to 30 min after injection of endotoxin. This showed that the pulmonary blood vessels of these rabbits were capable of developing hyperreacitivity to circulating ACh and that the hyperreactivity resulted in a pressor response in RVp. Failure to observe such a pressor response during AS in these animals indicates that hyperreactivity of pulmonary vasoconstrictor mechanisms to circulating ACh was absent during AS. It could be argued that massive pulmonary vasoconstriction during AS would have counteracted further constriction in response to ACh, thereby obscuring hyperreactivity to ACh. However, experiments with endotoxin in rabbits (7) have shown that hyperreactivity of pulmonary vasoconstrictor mechanisms to ACh will still result in a massive pressor response to i. v. ACh even when RVp is greatly elevated.

In 8 of 10 rabbits and in 6 of 7 guinea-pigs, ACh produced a smaller decrease in Ap during AS than before challenge. Our experiments do not rule out the objection that hyperreactivity of vasodilator mechanisms to ACh in the major circulation was obscured by the hypotension found during AS in rabbits, nor was hyperreactivity of vasodilator mechanisms to ACh after administration of endotoxin in rabbits demonstrated conclusively. However, in these animals endotoxin induced hyperreactivity of pulmonary cholinergic vasoconstrictor mechanisms to ACh. Since a decrease of sympathetic vasoconstrictor tone plays a more important role in dilatation of blood vessels than active cholinergic vasodilatation, we would not expect hyperreactivity of cholinergic vasodilator mechanisms to ACh present in blood and tissues to play a major role in the fall of Ap observed during AS in rabbits.

Absence of hyperreactivity of cholinergic vasodilator mechanisms to ACh in the major circulation was more evident in guinea-pigs, because ACh produced a smaller decrease in Ap during AS than before challenge, although in this species the Ap was elevated during shock.

The response in ITp to ACh was insignificant in both species, either before challenge or during AS.

Response to circulating histamine in any of the three parameters recorded was not significantly changed during AS either in rabbits or in guinea-pigs.

Our experiments indicate that hyperreactivity of pulmonary vascular and bronchial muscle to circulating ACh or histamine does not play a role in the pathogenesis of AS in rabbits or guinea-pigs.

Pseudomonas pseudomallei endotoxin was kindly provided by Dr. M. S. Redfearn of this laboratory.

Summary

The role of hyperreactivity of bronchial and vascular muscle to circulating acetylcholine (ACh) and to histamine in anaphylactic shock (AS) was investigated in rabbits and guinea-pigs. No increase in the responsiveness in intratracheal, systemic arterial and right ventricular blood pressures to ACh or histamine was observed in either species during AS. Endotoxin-induced hyperreactivity of pulmonary vasoconstrictor mechanisms to ACh in rabbits that had survived AS, demonstrated the capacity of pulmonary blood vessels to develop such a hyperreactivity. The results of the experiments indicate that hyperreactivity of bronchial or pulmonary vascular muscle to circulating ACh or to histamine does not play a role in the changes observed in arterial, right ventricular and intratracheal pressures during AS in rabbits and guinea-pigs.

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